

PATENT
USSN 10/087,473
Docket 090/003c

CLAIM AMENDMENTS

1. *(Currently amended)* A method for producing differentiated cells from a donor culture of undifferentiated primate pluripotent stem (pPS) cells, comprising a population of cells that is at least 75% homogeneous for a specific cell type, comprising:
 - a) preparing a suspension of pPS cells from the undifferentiated donor culture providing a suspension of undifferentiated human embryonic stem (hES) cells that is free of feeder cells;
 - b) replating plating and culturing the suspended cells on a solid surface so that they differentiate without forming embryoid bodies; and
 - c) harvesting differentiated cells from the solid surface, wherein at least 75% of the harvested cell population is homogeneous for said specific cell type.
2. *(Currently amended)* A method for obtaining differentiated cells from a donor culture of undifferentiated primate pluripotent stem (pPS) cells, comprising producing a population of cells that is at least 75% homogeneous for as specific cell type, comprising:
 - a) culturing the pPS cells undifferentiated hES cells on a solid surface in an environment essentially free of feeder cells;
 - b) changing medium used to culture the cells so that they differentiate before there is overgrowth or formation of colonies; and
 - c) harvesting differentiated cells from the solid surface, whereby at least 75% of the harvested cell population is homogeneous for said specific cell type.
3. CANCELLED
4. *(Currently amended)* The method of claim 1, wherein the donor culture is essentially free of feeder cells, which are replated hES cells are plated on a solid surface without any extracellular matrix.
5. *(Currently amended)* The method of claim 1, wherein the solid surface bears comprises a polycation.
6. *(Currently amended)* The method of claim 5, wherein the polycation is polyornithine or polylysine.
7. *(Currently amended)* The method of claim 1, wherein the cells are cultured after replating after plating, the cells are cultured in a medium containing a factor that promotes differentiation.

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8. The method of claim 7, wherein the factor is Brain Derived Neurotrophic Factor (BDNF) or Neutrotrophin-3 (NT-3).
9. The method of claim 2, wherein the changed medium is essentially free of fibroblast growth factor.
10. The method of claim 2, wherein the changed medium contains Brain Derived Neurotrophic Factor (BDNF) or Neutrotrophin-3 (NT-3).
11. The method of claim 2, wherein the changed medium contains noggin or follistatin.
12. CANCELLED
13. (Currently amended) The method of claim 12, wherein the precursor claim 1, wherein the differentiated cells are ectodermal cells.
14. The method of claim 13, wherein the precursor claim 1, wherein the differentiated cells are committed to the neuroectoderm lineage.
15. The method of claim 14, wherein the precursor claim 1, wherein the differentiated cells are cells of the mesoderm, endoderm or visceral endoderm.
16. CANCELLED
17. The method of claim 16, wherein the fully claim 1, wherein the differentiated cells are neurons or glial cells.
18. The method of claim 17, wherein at least ~10% of the cells staining positive for MAP-2 are also positive for tyrosine hydroxylase.
- 19 to 22. CANCELLED
23. (New) The method of claim 1, wherein the solid surface comprises an extracellular matrix component.
24. (New) The method of claim 2, wherein the changed medium contains retinoic acid.

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25. (New) The method of claim 2, wherein the changed medium contains DMSO or butyrate.
26. (New) The method of claim 2, wherein the changed medium contains hepatocyte growth factor.
27. (New) The method of claim 2, wherein the changed medium contains a glucocorticoid.
28. (New) The method of claim 2, wherein the differentiated cells are hepatocyte lineage cells.
29. (New) The method of claim 28, further comprising combining the cells of claim 28 with a test compound, determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with cellular toxicity or modulation.

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